

Assessment of theobromine-induced cytotoxicity in bladder cancer cell lines: Determination of IC₅₀ values step-by-step using RStudio

Teobrominin mesane kanseri hücre hatlarında sitotoksik etkisinin değerlendirilmesi: IC₅₀ değerlerinin adım adım RStudio kullanarak belirlenmesi

Elif ERCAN¹ (ID), Emine TERZİ¹ (ID), Tuba ÖZDEMİR SANCI² (ID), Beyza Ecem ÖZ BEDİR¹ (ID)

ABSTRACT

Objective: Cancer remains a significant global health issue, and the development of novel therapeutic strategies is crucial. Cisplatin, a widely used chemotherapeutic agent, is effective in treating various cancers but faces limitations due to resistance and side effects. Theobromine, a methylxanthine found in cocoa, has shown potential anticancer effects, but its efficacy and mechanisms remain less understood. This study investigates the cytotoxic effects of cisplatin and theobromine on bladder cancer cell lines, HTB9 and RT-112, and compares the use of RStudio and GraphPad Prism for IC₅₀ determination.

Methods: HTB9 and RT-112 cell lines were cultured and treated with cisplatin and theobromine at varying concentrations. Cell viability was assessed using WST-1 assay. Dose-response data were analyzed using RStudio and GraphPad Prism to calculate IC₅₀ values. RStudio's advanced statistical modeling and visualization capabilities, including the ggplot2 and drc packages,

ÖZET

Amaç: Kanser, küresel bir sağlık sorunudur ve yeni tedavi stratejilerinin geliştirilmesi gerekmektedir. Yaygın olarak kullanılan bir kemoterapötik ajan olan cisplatin, çeşitli kanserleri tedavi etmede etkili olsa da, tedavi direnci sorun oluşturmaktadır. Kakao içinde bulunan bir metilksantin olan teobromin ise potansiyel antikanser etkiler göstermiştir, ancak etkinliği ve mekanizmaları daha az anlaşılmıştır. Bu çalışmada, cisplatin ve teobrominin mesane kanseri hücre hatları HTB9 ve RT-112 üzerindeki sitotoksik etkileri araştırılmış ve IC₅₀ değerleri RStudio ile belirlenmiş, GraphPad Prism verileri ile karşılaştırılmıştır.

Yöntem: HTB9 ve RT-112 hücre hatları kültüre edilip, çeşitli konsantrasyonlarda cisplatin ve teobromin ile tedavi edilmiştir. Hücre canlılığı WST-1 testi ile değerlendirilmiştir. Doz-cevap verileri kullanılarak RStudio ve GraphPad Prism programlarında IC₅₀ değerleri hesaplanmıştır. RStudio'nun ggplot2 ve drc paketleri gibi gelişmiş istatistiksel modelleme

¹Ankara Yıldırım Beyazıt University, Faculty of Medicine, Department of Medical Biology, Ankara, Türkiye

²Ankara Yıldırım Beyazıt University, Faculty of Medicine, Department of Histology and Embryology, Ankara, Türkiye



İletişim / Corresponding Author : Beyza Ecem ÖZ BEDİR

Ankara Yıldırım Beyazıt Üniversitesi Tıp Fakültesi, Tıbbi Biyoloji AD., Ankara - Türkiye

E-posta / E-mail : beyzaecemozbedir@aybu.edu.tr

Geliş Tarihi / Received : 10.09.2024

Kabul Tarihi / Accepted : 23.02.2025

DOI ID : 10.5505/TurkHijyen.2026.65031

Ercan E, Terzi E, Özdemir Sancı T, Öz Bedir BE. Assessment of theobromine-induced cytotoxicity in bladder cancer cell lines: Determination of IC₅₀ values step-by-step using RStudio. Turk Hij Den Biyol Derg, 2026; 83(1): 3 - 14

were employed to assess dose-response relationships and IC_{50} values.

Results: Dose-response curves and IC_{50} values were generated for both cell lines and compounds. Cisplatin IC_{50} values were 9.398 μM and 2.018 μM for RT-112 and HTB9 cells respectively. Theobromine IC_{50} values were 15.253 μM and 3.78 μM for RT-112 and HTB9 cells respectively. Both RStudio and GraphPad Prism provided robust analyses, with RStudio offering detailed customization and advanced statistical modeling, while GraphPad Prism provided a user-friendly and efficient platform for IC_{50} determination.

Conclusion: The study highlights the comparative effectiveness of RStudio and GraphPad Prism in analyzing dose-response data. RStudio offers flexibility and precision for complex analyses, whereas GraphPad Prism is ideal for straightforward, efficient IC_{50} calculations. The findings suggest that researchers should choose between these tools based on their specific needs for customization, expertise, and ease of use in evaluating drug efficacy in cancer research.

Key Words: Bladder cancer, cisplatin, cytotoxicity, Rstudio, theobromine

ve görselleştirme yetenekleri kullanılarak doz-cevap ilişkileri ve IC_{50} değerleri değerlendirilmiştir.

Bulgular: Her iki hücre hattı ve bileşenler için doz-cevap eğrileri ve IC_{50} değerleri elde edilmiştir. Sisplatin IC_{50} değerleri sırasıyla RT-112 ve HTB9 hücreleri için 9.398 μM ve 2.018 μM olarak bulunmuştur. Teobromin IC_{50} değerleri ise sırasıyla RT-112 ve HTB9 hücreleri için 15.253 μM ve 3.78 μM olarak bulunmuştur. Hem RStudio hem de GraphPad Prism güçlü analizler sunmuş, RStudio ayrıntılı özelleştirme ve gelişmiş istatistiksel modelleme sunarken, GraphPad Prism IC_{50} hesaplamaları için verimli ve kullanıcı dostu bir platform olduğu görülmüştür.

Sonuç: Bu çalışma, doz-cevap verilerinin analizinde RStudio ve GraphPad Prism'in karşılaştırmalı etkinliğini vurgulamıştır. RStudio, karmaşık analizler için esneklik ve hassasiyet sunarken, GraphPad Prism IC_{50} hesaplamaları için kolaylık açısından ideal bulunmuştur. Bulgular, araştırmacıların ilaç etkinliğini değerlendirmede bu araçlar arasında özel ihtiyaçlarına göre seçim yapmaları gerektiğini önermektedir.

Anahtar Kelimeler: Mesane kanseri, sisplatin, sitotoksosite, RStudio, teobromin

INTRODUCTION

Cancer continues to be a major worldwide health concern, requiring ongoing research and development of new treatment approaches. Bladder cancer remains one of the most prevalent and challenging malignancies worldwide, necessitating ongoing research to develop effective therapeutic strategies (1). The RT-112 cell line is a widely utilized model for studying human urothelial carcinoma (2,3). This bladder cancer cell line derived from a female patient in 1973. This cell line originates from transitional cell carcinoma of the bladder. RT-112 cells exhibit an epithelioid morphology, growing in

adherent monolayers (4). The HTB9 cell line derived from human urothelial carcinoma, has emerged as a critical model for studying the pathophysiology of bladder cancer. This cell line is characterized by its origin from a high-grade transitional cell carcinoma, providing a representative model for investigating the aggressive nature of bladder cancer. It retains many features of the original tumor, including its invasive properties and resistance to conventional therapies, making it a valuable tool for preclinical research (5-7). These cell lines are extensively used to explore various aspects of bladder cancer biology, including tumorigenesis, drug resistance, and the efficacy of new therapeutic compounds. They provide a relevant

platform for evaluating therapeutic agents aimed at treating bladder cancer (8,9).

Cisplatin, a platinum-based chemotherapeutic agent, has been a cornerstone in the treatment of various cancers due to its ability to induce DNA cross-links, thereby disrupting DNA replication and triggering apoptotic pathways (10-12). Despite its efficacy, Cisplatin's clinical use is limited by the development of resistance and significant side effects, highlighting the need for complementary or alternative therapeutic approaches (13). Theobromine, a methylxanthine found predominantly in cocoa and chocolate, has been recognized for its stimulant and antioxidant properties (14,15). Emerging research suggests that theobromine might possess anticancer potential, though its effects and mechanisms are less well-characterized compared to established chemotherapeutic agents (16-18). Theobromine has demonstrated cytotoxic effects in various cancer models, potentially through mechanisms involving apoptosis and cell cycle regulation (19,20). Theobromine exerts its anti-cancer effects by inducing apoptosis, inhibiting angiogenesis and cell proliferation and targeting molecules such as EGFR, which is overexpressed in cancer (21,22).

The Water Soluble Tetrazolium-1 (WST-1) assay is a widely employed colorimetric method for evaluating cell viability and proliferation. This assay is based on the reduction of tetrazolium salts to formazan dyes, which provides an indirect measure of cellular metabolic activity and, cell viability (23,24). The accurate assessment of dose-response relationships is crucial in evaluating the efficacy and toxicity of new therapeutic agents, making the determination of appropriate dosing levels a key aspect of pharmacological research (25).

The analysis of dose-response data can be complex, requiring sophisticated statistical and graphical tools to accurately interpret the results. GraphPad Prism and RStudio are two powerful tools commonly used for this purpose. The Rstudio interface is an integrated development environment

for using the R programming language. GraphPad Prism offers robust capabilities for visualizing data and performing nonlinear regression analyses, such as IC_{50} (half-maximal inhibitory concentration) calculations. In contrast, RStudio provides extensive functionalities for statistical modeling and data manipulation, allowing for more customized and detailed analyses. GraphPad Prism is widely used for its strengths in data visualization and to derive key dose-response parameters.

In this context, our study aimed to evaluate the cytotoxic effects of cisplatin and theobromine on the HTB9 and RT-112 bladder carcinoma cell lines. By providing insights into the efficacy of these compounds, this research could contribute to the development of improved treatment regimens for bladder cancer. We used WST-1 assay to investigate cell viability at various dosages and used RStudio (version 1.1.463) to analyze and interpret the data. RStudio was used to perform advanced statistical modeling capabilities and comprehensive data analysis. We aimed to expand the use of RStudio, improve the precision of our dose-response assessments and gain deeper insights into the effects of various treatments. For this purpose we compared the IC_{50} values obtained from GraphPad Prism software and RStudio.

This study investigates the cytotoxic effects of cisplatin and theobromine on bladder cancer cell lines, HTB9 and RT-112, and compares the use of RStudio and GraphPad Prism for IC_{50} determination.

MATERIAL and METHOD

Cell Culture

Human invasive bladder cancer HTB9 and human non-invasive bladder cancer RT-112 cell lines were cultured in RPMI-1640 medium containing 10% Fetal Bovine Serum and 1% penicillin/streptomycin at 37°C and 5% CO₂ in two dimensions.

Theobromine was acquired from Sigma-Aldrich (catalog number: T4500) and dissolved in dimethyl

sulfoxide (DMSO, Merck, Saint Louis, MO, USA) to have a 1 mM stock solution. Cisplatin (European Pharmacopoeia) was prepared with DMSO to obtain a 500 μM stock solution.

Cell proliferation and differentiation

For measurement of cell viability and proliferation, RT-112 and HTB9 cells were seeded at 5×10^4 cells/well in 96-well plates. These cells were divided into three groups. Cells treated with 0, 5, 10, 25, 50, 100 or 200 μM theobromine (Sigma-Aldrich-T4500); 0, 1, 2.5, 5, 10, 25 or 50 μM cisplatin (European Pharmacopoeia) (as positive control) and untreated control group (negative control). To eliminate the effects from DMSO, the concentrations were added to the negative control wells at a rate where theobromine and cisplatin were dissolved along with the medium.

The colorimetric WST-1 (5 mg/mL, Cayman Chemical) for measuring cell viability and proliferation was performed at 24 and 48 hours for RT-112 and HTB9 cells. 10 μl of WST-1 reagent will be added to all wells and after 4 hours of incubation, absorbance was read on a microplate reader (BioTek Epoch Microplate Spectrophotometer) at 450 nm. IC_{50} value will be calculated using RStudio program (version 1.1.463). All experiments were performed three times.

Determination of IC_{50} Values Using GraphPad Prism

To create a dose-response graph for RT-112 and HTB9 cells that were treated with cisplatin and theobromine at different doses over 24 and 48 hours using GraphPad Prism, follow these steps (Figure 1a, Figure 1b, Figure 2a and Figure 2b).

Prepare your data

We converted our data into a table with concentrations (independent variable, X-axis) and corresponding responses (dependent variable, Y-axis, such as % inhibition or viability). And ensured that the datas are normalized (100% for the highest effect).

Steps in GraphPad Prism

We created a new data table and selected XY as the type of data table and graph. We inputted our datas into the table. The X values were the concentrations (log scale), and Y values were the responses.

Select the analysis

Go to Analyze > Nonlinear regression (curve fit) > Dose-response - Inhibition > Log(inhibitor) vs. response -- Variable slope

View the results

The IC_{50} value were displayed in the results table, along with other parameters.

Determination of IC_{50} Values Using RStudio

To create a dose-response graph for RT-112 and HTB9 cells that were treated with cisplatin and theobromine at different doses over 24 and 48 hours using RStudio, follow these steps. We used the ggplot2 package to visualize the data.

Install and Load Required Packages

First, we installed the “ggplot2” package in the RStudio interface as follows:

```
install.packages("ggplot2")
library(tidyr)
library(dplyr)
library(ggplot2)
```

Prepare the Data

We organized our data into a data frame to create a dose-response graph for RT-112 cells treated with cisplatin at different doses over 24 and 48 hours using RStudio:

```
# Define the doses and responses for RT-112 cells
# treated with cisplatin
doses <- c(0, 1, 2.5, 5, 10, 25, 50)
response_24h_replicates <- data.frame(Dose =
doses,
Rep1 = c(100.0, 98.2, 96.1, 93.4, 46.3, 8.5, 6.2),
Rep2 = c(99.8, 97.7, 95.3, 93.6, 45.4, 9.1, 5.8),
Rep3 = c(100.1, 99.3, 96.8, 92.7, 46.8, 7.9, 6.4))
```

```

response_48h_replicates <- data.frame(Dose =
doses,
  Rep1 = c(100.0, 89.8, 83.5, 70.2, 30.1, 6.3, 4.2),
  Rep2 = c(99.7, 90.2, 83.2, 69.7, 29.8, 6.0, 4.4),
  Rep3 = c(100.3, 90.5, 82.8, 70.0, 30.4, 6.1, 4.1))
# Transform data to long format
response_24h_long <- response_24h_replicates
%>% pivot_longer(-Dose, names_to = "Repeat",
values_to = "Viability") %>% mutate(Time = "24
Hours")
response_48h_long <- response_48h_replicates
%>% pivot_longer(-Dose, names_to = "Repeat",
values_to = "Viability") %>% mutate(Time = "48
Hours")
# Combine datasets
combined_data <- rbind(response_24h_long,
response_48h_long)
# Calculate means and standard errors
summary_data <- combined_data %>%
group_by(Dose, Time) %>% summarize(Mean =
mean(Viability), SE = sd(Viability) / sqrt(n()), .groups
= "drop" )

```

We organized our data into a data frame to create a dose-response graph for HTB9 cells treated with cisplatin at different doses over 24 and 48 hours using RStudio:

```

# Define the doses and responses for HTB9 cells
treated with cisplatin
doses <- c(0, 1, 2.5, 5, 10, 25, 50)
response_24h_replicates <- data.frame(Dose =
doses,
  Rep1 = c(98.7, 95.9, 38.2, 19.4, 14.5, 12.1, 9.3),
  Rep2 = c(104.5, 91.7, 41.5, 18.1, 13.6, 12.4,
8.6),
  Rep3 = c(102.3, 91.7, 40.4, 19.9, 13.6, 11.9,
9.0))
response_48h_replicates <- data.frame(Dose =
doses,
  Rep1 = c(98.9, 89.5, 32.4, 17.5, 10.2, 3.9, 3.2),
  Rep2 = c(99.7, 90.3, 33.9, 17.1, 10.1, 4.1, 3.3),

```

```

  Rep3 = c(100.4, 90.8, 34.2, 16.9, 10.0, 3.8, 3.1))
# Transform data to long format
response_24h_long <- response_24h_replicates
%>% pivot_longer(-Dose, names_to = "Repeat",
values_to = "Viability") %>% mutate(Time = "24
Hours")
response_48h_long <- response_48h_replicates
%>% pivot_longer(-Dose, names_to = "Repeat",
values_to = "Viability") %>% mutate(Time = "48
Hours")
# Combine datasets
combined_data <- rbind(response_24h_long,
response_48h_long)
# Calculate means and standard errors
summary_data <- combined_data %>%
group_by(Dose, Time) %>% summarize(Mean =
mean(Viability), SE = sd(Viability) / sqrt(n()), .groups
= "drop" )

```

We organized our data into a data frame to create a dose-response graph for RT-112 cells treated with theobromine at different doses over 24 and 48 hours using RStudio:

```

# Define the doses and responses for RT-112 cells
treated with theobromine
doses <- c(0, 5, 10, 25, 50, 100, 200)
response_24h_replicates <- data.frame(Dose =
doses,
  Rep1 = c(98.75, 100.33, 90.04, 19.19, 13.52,
11.59, 8.60),
  Rep2 = c(103.66, 96.97, 89.83, 18.09, 14.66,
12.40, 8.74),
  Rep3 = c(96.82, 92.96, 86.28, 19.05, 13.90,
11.75, 9.10))
response_48h_replicates <- data.frame(Dose =
doses,
  Rep1 = c(96.39, 88.13, 43.41, 32.86, 25.71,
18.43, 8.01),
  Rep2 = c(100.92, 85.92, 44.47, 31.91, 23.91,
19.85, 8.37),
  Rep3 = c(103.08, 88.24, 42.23, 33.61, 24.85,

```

```

18.28, 8.00))
# Transform data to long format
response_24h_long <- response_24h_replicates
%>% pivot_longer(-Dose, names_to = "Repeat",
values_to = "Viability") %>% mutate(Time = "24
Hours")
response_48h_long <- response_48h_replicates
%>% pivot_longer(-Dose, names_to = "Repeat",
values_to = "Viability") %>% mutate(Time = "48
Hours")
# Combine datasets
combined_data <- rbind(response_24h_long,
response_48h_long)
# Calculate means and standard errors
summary_data <- combined_data %>%
group_by(Dose, Time) %>% summarize(Mean =
mean(Viability), SE = sd(Viability) / sqrt(n()), .groups
= "drop" )
We organized our data into a data frame to create
a dose-response graph for HTB9 cells treated with
theobromine at different doses over 24 and 48 hours
using RStudio:
# Define the doses and responses for HTB9 cells
treated with theobromine
doses <- c(0, 5, 10, 25, 50, 100, 200)
response_24h_replicates <- data.frame(Dose =
doses,
Rep1 = c(98.75, 48.47, 24.85, 20.42, 15.50,
11.62, 9.93),
Rep2 = c(104.51, 46.35, 26.95, 19.04, 14.57,
11.77, 9.79),
Rep3 = c(102.32, 46.35, 26.26, 20.94, 14.52,
12.03, 10.11))
response_48h_replicates <- data.frame(Dose =
doses,
Rep1 = c(96.39, 37.83, 23.03, 16.17, 7.31, 1.96,
1.99),
Rep2 = c(97.92, 39.08, 23.21, 15.47, 7.33, 1.92,
1.92),
Rep3 = c(98.66, 36.86, 21.96, 15.30, 7.22, 2.04,

```

```

2.00))
# Transform data to long format
response_24h_long <- response_24h_replicates
%>% pivot_longer(-Dose, names_to = "Repeat",
values_to = "Viability") %>% mutate(Time = "24
Hours")
response_48h_long <- response_48h_replicates
%>% pivot_longer(-Dose, names_to = "Repeat",
values_to = "Viability") %>% mutate(Time = "48
Hours")
# Combine datasets
combined_data <- rbind(response_24h_long,
response_48h_long)
# Calculate means and standard errors
summary_data <- combined_data %>%
group_by(Dose, Time) %>% summarize(Mean =
mean(Viability), SE = sd(Viability) / sqrt(n()), .groups
= "drop" )

```

Plot the Data

We used ggplot2 to create the graph:

To create a dose-response graph for RT-112 cells treated with cisplatin at different doses over 24 and 48 hours using RStudio (Figure 3a):

```

# Create the plot for RT-112 cells treated with
cisplatin
ggplot(summary_data, aes(x = Dose, y = Mean,
color = Time, shape = Time)) + geom_line() + geom_
point(size = 3) + geom_errorbar(aes(ymin = Mean - SE,
ymax = Mean + SE), width = 0.2) + scale_x_log10() +
labs(x = "Dose (µM)", y = "Viability (%)", title = "Dose-
Response with Replicates (24h & 48h) of Cisplatin on
RT-112 Cells") + theme_minimal() + theme(legend.
position = "bottom") + scale_color_manual(values =
c("24 Hours" = "blue", "48 Hours" = "red")) + scale_
shape_manual(values = c(16, 17))

```

To create a dose-response graph for HTB9 cells treated with cisplatin at different doses over 24 and 48 hours using RStudio (Figure 3b):

```

# Create the plot for HTB9 cells treated with
cisplatin

```

```
ggplot(summary_data, aes(x = Dose, y = Mean,
color = Time, shape = Time)) + geom_line() + geom_
point(size = 3) + geom_errorbar(aes(ymin = Mean - SE,
ymax = Mean + SE), width = 0.2) + scale_x_log10() +
labs(x = "Dose ( $\mu$ M)", y = "Viability (%)", title = "Dose-
Response with Replicates (24h & 48h) of Cisplatin on
HTB9 Cells") + theme_minimal() + theme(legend.
position = "bottom") + scale_color_manual(values =
c("24 Hours" = "blue", "48 Hours" = "red")) + scale_
shape_manual(values = c(16, 17))
```

To create a dose-response graph for RT-112 cells treated with theobromine at different doses over 24 and 48 hours using RStudio (Figure 4a):

```
# Create the plot for RT-112 cells treated with
theobromine
```

```
ggplot(summary_data, aes(x = Dose, y = Mean,
color = Time, shape = Time)) + geom_line() + geom_
point(size = 3) + geom_errorbar(aes(ymin = Mean - SE,
ymax = Mean + SE), width = 0.2) + scale_x_log10() +
labs(x = "Dose ( $\mu$ M)", y = "Viability (%)", title = "Dose-
Response with Replicates (24h & 48h) of Theobromine
on RT-112 Cells") + theme_minimal() + theme(legend.
position = "bottom") + scale_color_manual(values =
c("24 Hours" = "blue", "48 Hours" = "red")) + scale_
shape_manual(values = c(16, 17))
```

To create a dose-response graph for HTB9 cells treated with theobromine at different doses over 24 and 48 hours using RStudio (Figure 4b):

```
# Create the plot for HTB9 cells treated with
theobromine
```

```
ggplot(summary_data, aes(x = Dose, y = Mean,
color = Time, shape = Time)) + geom_line() + geom_
point(size = 3) + geom_errorbar(aes(ymin = Mean - SE,
ymax = Mean + SE), width = 0.2) + scale_x_log10() +
labs(x = "Dose ( $\mu$ M)", y = "Viability (%)", title = "Dose-
Response with Replicates (24h & 48h) of Theobromine
on HTB9 Cells") + theme_minimal() + theme(legend.
position = "bottom") + scale_color_manual(values =
c("24 Hours" = "blue", "48 Hours" = "red")) + scale_
shape_manual(values = c(16, 17))
```

Calculate the IC₅₀ value

We installed the “drc” package in the RStudio interface as follows:

```
install.packages("drc")
library(drc)
```

We generated a dose-response curve using the drc package to calculate the IC₅₀ value for all 24 hour values:

```
# Fit a dose-response model
```

```
model <- drm(response_24h ~ doses, data = data,
fct = LL.4())
```

```
# Calculate IC50
```

```
ic50_value <- ED(model, 50, interval = "delta")
print(ic50_value)
```

Code Explanations

aes(x = Dose, y = Viability, color = Time, shape = Time): Maps dose to the x-axis, viability to the y-axis, and distinguishes between 24 and 48 hours using color and shape.

geom_line(): Adds lines connecting the data points.

geom_point(size = 3): Adds points at each data point with size 3.

scale_x_log10(): Sets the x-axis to a logarithmic scale to better visualize the dose-response relationship.

labs(): Adds labels for the x and y axes and the plot title.

theme_minimal(): Applies a minimal theme to the plot.

theme(legend.position = "bottom"): Places the legend at the bottom of the plot.

scale_color_manual() and scale_shape_manual(): Customizes the colors and shapes used for the different time points.

The pivot_longer: Is used to convert the wide-format data into long-format data. Each replicate is placed in a separate row, making it easier to calculate statistics and plot.

geom_errorbar: Adds error bars to the plot, representing the variability (mean \pm SE) of each data point.

RESULTS

Dose-response curves and IC_{50} values were generated for both cell lines and compounds.

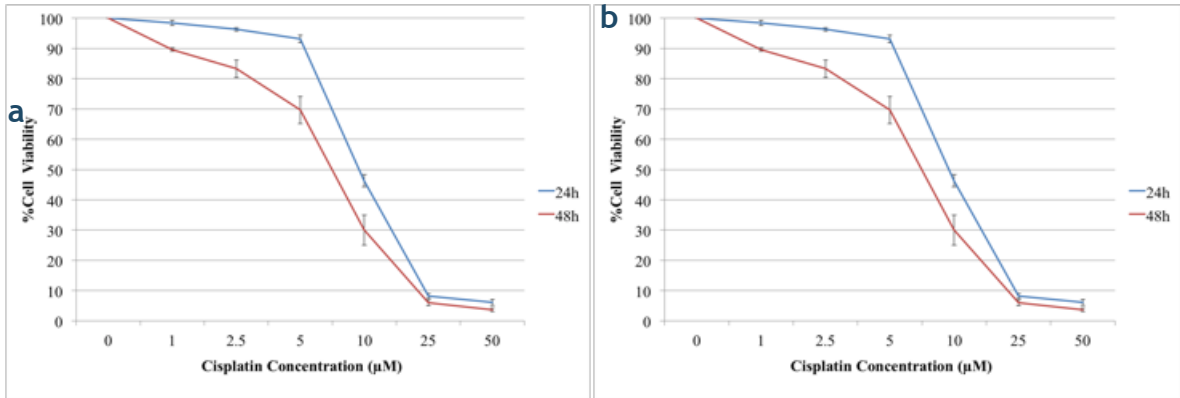


Figure 1. a) Dose-response graph for RT-112 cells treated with cisplatin at different doses over 24 and 48 hours using GraphPad Prism. b) Dose-response graph for HTB9 cells treated with cisplatin at different doses over 24 and 48 hours using GraphPad Prism.

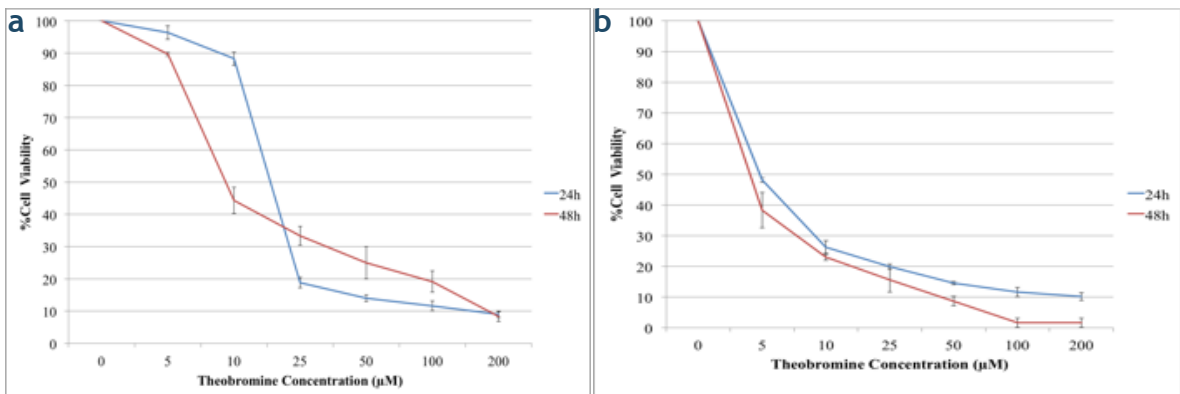


Figure 2. a) Dose-response graph for RT-112 cells treated with theobromine at different doses over 24 and 48 hours using GraphPad Prism. b) Dose-response graph for HTB9 cells treated with theobromine at different doses over 24 and 48 hours using GraphPad Prism.

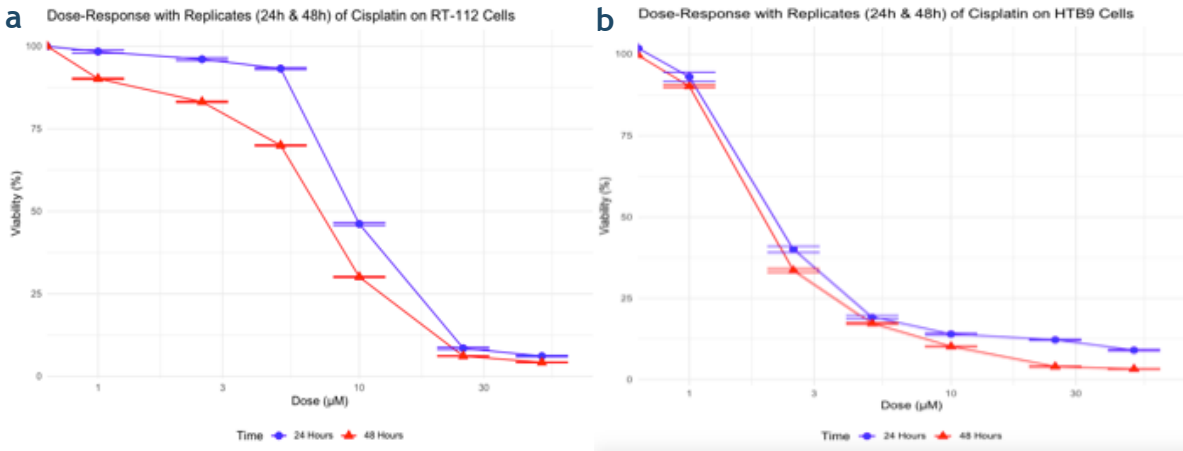


Figure 3. a) Dose-response graph for RT-112 cells treated with cisplatin at different doses over 24 and 48 hours using RStudio. b) Dose-response graph for HTB9 cells treated with cisplatin at different doses over 24 and 48 hours.

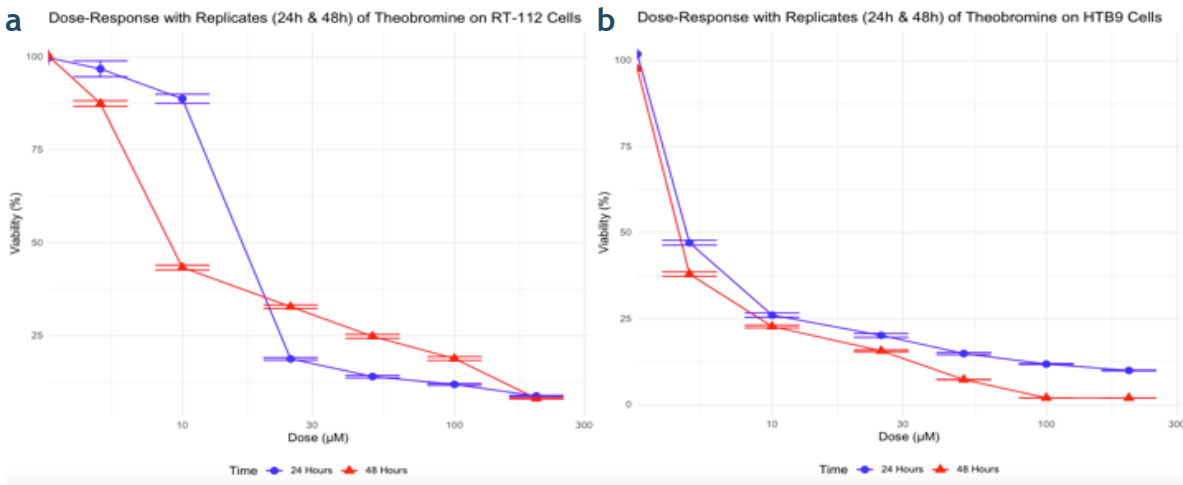


Figure 4. a) Dose-response graph for RT-112 cells treated with theobromine at different doses over 24 and 48 hours. b) Dose-response graph for HTB9 cells treated with theobromine at different doses over 24 and 48 hours.

IC₅₀ values

IC₅₀ values for each cell line were calculated based on 24-hour viability data using the “drc” package in RStudio. The results are presented in the tables below (Table 1). Cisplatin IC₅₀ values were 9.398 µM and 2.018 µM for RT-112 and HTB9 cells respectively. Theobromine IC₅₀ values were 15.253

µM and 3.78 µM for RT-112 and HTB9 cells respectively.

We determined the IC₅₀ values for each cell line and each dose using the GraphPad Prism software (version 9.1.0) as follows; for the RT-112 cell line 9.8 µM for cisplatin and 17.19 µM for theobromine, for the HTB9 cell line 2.4 µM for cisplatin and 3.3 µM for theobromine.

Table 1. Estimated effective doses of inhibitors on RT-112 and HTB9

Cell line / Inhibitor	e:1:50	Estimate	Standard Error	Lower	Upper
RT-112 / Cisplatin	μM	9.398	0.172	8.851	9.944
HTB9 / Cisplatin	μM	2.018	0.109	1.671	2.364
RT-112 / Theobromine	μM	15.253	0.822	12.636	17.870
HTB9 / Theobromine	μM	3.780	0.518	2.131	5.429

DISCUSSION

There is no study in the literature showing the IC_{50} values of theobromine in cancer cell lines especially in bladder cancer cells. However the IC_{50} values of theobromine and its derivatives are explained in a few study. Bedir et. al (17) found the IC_{50} value of theobromine 16.02 μM for 24 h and 10.76 μM for 48 h in A549 lung cancer cell line.

We determined the IC_{50} values of RT-112 cell line 9.8 μM for cisplatin and 17.19 μM for theobromine in GraphPad Prism software. It is found to be 9.398 μM for cisplatin and 15.253 μM for theobromine in RStudio. Also, we determined the IC_{50} values of HTB9 cell line 2.4 μM for cisplatin and 3.3 μM for theobromine in GraphPad Prism software. It is found to be 2.018 μM for cisplatin and 3.780 μM for theobromine in RStudio.

Our findings are consistent with the literature. Rabenstein et al. found cisplatin IC_{50} value of 610.5 nM at 24 hours in HTB9 cell line in their study (26). Höhn et al. reported that the cisplatin IC_{50} value in RT-112 cell line was 10.7 μM at 72 hours (27).

This study provides a comparative analysis of RStudio and GraphPad Prism in determining IC_{50} values, assessing their sensitivity and accuracy in evaluating drug efficacy and potency. Our findings highlight the strengths and limitations of each tool in the context of dose-response analysis.

RStudio, leveraging the R programming language, offers significant flexibility and precision in IC_{50} calculations. Its sensitivity is enhanced by the ability to apply a wide range of statistical models and customize analyses according to specific experimental

conditions. Advanced packages in R, such as drc and ggplot2, allow for sophisticated modeling and detailed visualization of dose-response curves. This flexibility, however, requires a certain level of programming expertise and can be time-consuming, which might be a barrier for users with less statistical experience. In contrast, GraphPad Prism excels in providing a user-friendly platform with built-in tools specifically designed for IC_{50} analysis. Its sensitivity is demonstrated through streamlined processes for fitting dose-response curves and determining IC_{50} values with minimal manual input. Prism's intuitive interface and comprehensive graphical outputs facilitate quick and accurate assessments, making it highly accessible for researchers seeking efficiency and ease of use. However, the proprietary nature of Prism may limit its adaptability for more complex or non-standard analyses.

In conclusion; RStudio and GraphPad Prism both offer valuable capabilities for IC_{50} determination, each with its distinct advantages. RStudio is well-suited for users requiring detailed customization and advanced statistical modeling, while GraphPad Prism is ideal for those prioritizing straightforward, efficient analysis and ease of use. The choice between these tools should be guided by the researcher's specific needs for flexibility, expertise, and user-friendliness.

Researchers can copy and paste these codes into an R script or the R console in RStudio and run it. This will generate a plot showing the dose-response relationship for different inhibitor treatment in various cancer cell lines.

ETHICS COMMITTEE APPROVAL

* This study does not require Ethics Committee Approval.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Ercan E, Sımsek E, Guler OO, Canda AE, Atmaca AF, Carhan A. Determination of circulating tumor cells by flow cytometry in the bladder cancer patients. *Urol Androl - Open J*, 2018;2(1):25-30.
2. Pasquale V, Ducci G, Campioni G, Ventrici A, Assalini C, Busti S, et al. Profiling and targeting of energy and redox metabolism in grade 2 bladder cancer cells with different invasiveness properties. *Cells*, 2020;9(12):2669.
3. Berndt-Paetz M, Han S, Weimann A, Reinhold A, Nürnbergger S, Neuhaus J. Cell line-based human bladder organoids with bladder-like self-organization—a new standardized approach in bladder cancer research. *Biomedicines*, 2023;11(11):2958.
4. DSMZ CellDive. Available from: <https://www.dsmz.de/collection/catalogue/details/culture/ACC-418>. Accessed July 01, 2024.
5. Vautrin N, Dahyot S, Leoz M, Caron F, Grand M, Feldmann A, et al. Are Escherichia coli causing recurrent cystitis just ordinary uropathogenic E. coli (UPEC) strains? *bioRxiv Prepr Serv Biol*, 2023:2023.11.08.566351.
6. Iskender B, Izgi K, Karaca H, Canatan H. Myrtucommulone-A treatment decreases pluripotency- and multipotency-associated marker expression in bladder cancer cell line HTB-9. *J Nat Med*, 2015;69(4):543-54.
7. Choudhary D, Clement JM, Choudhary S, Voznesensky O, Pilbeam CC, Woolbright BL, et al. SATB1 and bladder cancer: Is there a functional link? *Urol Oncol Semin Orig Investig*, 2018;36(3):93.e13-93.e21.
8. Sancı TO, Terzi E, Oz Bedir BE, Gumustas M, Aydin T, Cakir A. Effect of herniarin on cell viability, cell cycle, and erk protein levels in different stages of bladder cancer cells. *Chem Biodivers*, 2024;14;21(3).
9. Railkar R, Krane LS, Li QQ, Sanford T, Siddiqui MR, Haines D, et al. Epidermal growth factor receptor (EGFR)-targeted photoimmunotherapy (PIT) for the treatment of EGFR-expressing bladder cancer. *Mol Cancer Ther*, 2017;16(10):2201-14.
10. Tchounwou PB, Dasari S, Noubissi FK, Ray P, Kumar S. Advances in our understanding of the molecular mechanisms of action of cisplatin in cancer therapy. *J Exp Pharmacol*, 2021;Volume 13:303-28.

11. Kiss RC, Xia F, Acklin S. Targeting DNA damage response and repair to enhance therapeutic index in cisplatin-based cancer treatment. *Int J Mol Sci*, 2021;22(15):8199.
12. Çiçek B, Taghizadehghalehjoughi A, Yildirim S, Eser G, Gül M, Kantarci M, et al. Oxytocin administration improves DNA damage and total oxidative stress parameters in vincristine and cisplatin-induced cortical neuron toxicity. *Turkish Bull Hyg Exp Biol*, 2022;79(4):730-9.
13. Shruthi S, Bhasker Shenoy K. Cisplatin resistance in cancer therapy: causes and overcoming strategies. *Chemistry Select*, 2024;9(25).
14. Cortez D, Quispe-Sanchez L, Mestanza M, Oliva-Cruz M, Yoplac I, Torres C, et al. Changes in bioactive compounds during fermentation of cocoa (*Theobroma cacao*) harvested in Amazonas-Peru. *Curr Res Food Sci*, 2023;6:100494.
15. Martinez-Pinilla E, Onatibia-Astibia A, Franco R. The relevance of theobromine for the beneficial effects of cocoa consumption. *Front Pharmacol*, 2015;6.
16. Eissa IH, Yousef RG, Elkady H, Elkaeed EB, Alsouk AA, Husein DZ, et al. Design, semi-synthesis, anti-cancer assessment, docking, MD simulation, and DFT studies of novel theobromine-based derivatives as VEGFR-2 inhibitors and apoptosis inducers. *Comput Biol Chem*, 2023;107:107953.
17. Cadona FC, Dantas RF, de Mello GH, Silva-Jr FP. Natural products targeting into cancer hallmarks: An update on caffeine, theobromine, and (+)-catechin. *Crit Rev Food Sci Nutr*, 2022;62(26):7222-41.
18. Bedir BO, Sancı TO, Ercan E, Sezginer EK, E. Terzi. In vitro anticancer effect of theobromine in A549 non-small cell lung cancer cells. *Int J Med Biochem*, 2024;7(3):143-9.
19. David Osarieme, E, Modupe, DT, Oluchukwu OP. The anticancer activity of caffeine - a review. *Arch Clin Biomed Res*, 2019;3(5): 326-42.
20. Shojaei-Zarghani S, Rafrat M, Yari Khosroushahi A, Sheikh-Najafi S. Effectiveness of theobromine on inhibition of 1,2-dimethylhydrazine-induced rat colon cancer by suppression of the Akt/GSK3B/B-catenin signaling pathway. *J Funct Foods*, 2020;75:104293.
21. Eissa IH, Yousef RG, Elkady H, Alsouk AA, Alsouk BA, Husein DZ, et al. A New anticancer semisynthetic theobromine derivative targeting EGFR protein: CADD study. *Life*, 2023;13(1):191.
22. Eissa IH, Yousef RG, Elkaeed EB, Alsouk AA, Husein DZ, Ibrahim IM, et al. Anticancer derivative of the natural alkaloid, theobromine, inhibiting EGFR protein: computer-aided drug discovery approach. Al-Karmalawy AA, editor. *PLoS One*, 2023;18(3):e0282586.
23. Sari C. A comparative study of MTT and WST-1 assays in cytotoxicity analysis. *Haydarpaşa Numune Train Res Hosp Med J*, 2019; 2021; 61(3): 281-8.
24. Taskin A, Ulusal H, Taskin S, Tarakcioglu M. Tetrazolium-based cytotoxicity tests may not always reflect accurate results. *Harran Üni Tıp Fak Derg*, 2020;17(1):6-12.
25. Özalper B, Özdemir Sancı T, Özgüner H. Antiproliferative effects of vitamin K2 in osteosarcoma cells: comparison of different cytotoxicity analyzes. *SDÜ Tıp Fak Derg*, 2023;30(1):1-8.
26. Rabenstein J, Fischer DC, Hakenberg OW, Jahn D, Rutz W, Hohn A, et al. Monitoring cytotoxicity of gemcitabine and cisplatin in T24 bladder cancer cells by the use of F-18-FDG and F-18-FMC. *Int J Clin Exp Med*, 2017;10(3):4556-64.
27. Höhn A, Krüger K, Skowron MA, Bormann S, Schumacher L, Schulz WA, et al. Distinct mechanisms contribute to acquired cisplatin resistance of urothelial carcinoma cells. *Oncotarget*, 2106;7(27):41320-35.